

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
TRIS (HYDROXYMETHYL) NITROMETHANE

Chemical Code # 001105, Tolerance # 50275

SB 950 # 928

Original date: April 12, 2002

Revised August 19, 2003

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no study on file.
Subchronic, rat, dermal:	No data gap, no adverse effect
Chronic toxicity, dog:	Data gap, no study on file.
Oncogenicity, rat:	Data gap, no study on file.
Oncogenicity, mouse:	Data gap, no study on file.
Reproduction, rat:	Data gap, no study on file.
Teratology, rat:	No data gap, possible adverse effect (resorptions).
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time.

Toxicology one-liners are attached.

All record numbers through 205489 were examined.

* indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T030819

Revised by: J. Kishiyama and Gee, April 12, 2002, revised by Gee, 8/19/03

Tris(hydroxymethyl)nitromethane is an antimicrobial used in disinfectants and industrial preservatives. The "Reregistration Eligibility Document: Tris(hydroxymethyl)nitromethane" was issued in September, 1993, by USEPA. This document states that there is no tolerance or exemption from tolerance as no dietary exposure is expected. The major concern was with the formaldehyde that is generated.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

Subchronic, rat, dermal:

** 50275 - 004 073175 Naas, D. J. "TRIS NITRO® Brand of 2-Hydroxy-2-nitro-1,3-propanediol: 90-Day Dermal Toxicity Study in Rats." (WIL Research Laboratories, Project No. WIL-129005, February 22, 1989.) TRIS NITRO®, 100% ai, was administered dermally (6 hours/day, 5 days/week for 13 weeks) at doses of 0 (deionized water), 250, 500, or 1000 mg/kg/day to 15 Crl:CD®BR rats/sex/group. Each dose was weighed, moistened to a paste with water and applied under occlusive dressing. There were no treatment-related effects on clinical signs (other than yellow staining of the skin at the site of application), hematology, clinical chemistry, ophthalmology, urinalysis or histopathology. NOEL = 1000 mg/kg/day. ACCEPTABLE with no adverse effect. (Kishiyama and Gee, 4/4/02).

CHRONIC TOXICITY, RAT

No study submitted.

CHRONIC TOXICITY, DOG

No study submitted.

ONCOGENICITY, RAT

No study submitted.

ONCOGENICITY, MOUSE

No study submitted.

REPRODUCTION, RAT

No study submitted.

TERATOLOGY, RAT

50275 - 005 073176 Nemec, M. D. "TRIS NITRO® Brand of 2-hydroxymethyl-2-nitro-1,3-propanediol: A Range-Finding Teratology Study in Rats." (WIL Research Laboratories, Project No. WIL-129001, 8/23/88) TRIS NITRO®, 100% ai, was administered via gavage at doses of 0 (deionized water, 10 ml/kg), 250, 500, 750, 1000, or 1500 mg/kg/day during gestation days 6 through 15 to five mated female Sprague-Dawley Crl:CD®BR rats/group. Mortality was 1/5 and 3/5 for 1000 and 1500 mg/kg/day groups. The author considered the mortality to be treatment related at the two highest doses. At a dose level of 750 mg/kg/day, there were no significant effects. No fetal effects on litter size, pre- or post-implantation loss. Fetal weights were not recorded. Supplemental range-finding study. (Kishiyama and Gee, 4/3/02).

**** 50275 - 005 073180** Nemec, M. D. "TRIS NITRO[®] Brand of 2-hydroxymethyl-2-nitro-1,3-propanediol: A Teratology Study in Rats." (WIL Research Laboratories, Project No. WIL-129002, 2/2/89) TRIS NITRO[®], 100% ai, was administered via gavage at doses of 0 (deionized water), 50, 375, or 750 mg/kg/day during gestation days 6 through 15 to twenty-five mated female Sprague-Dawley CrI:CD®BR rats/group. Tris Nitro at the high dosage resulted in increased mortality with 7/25 deaths days 9 - 11 of gestation. Six of these dams had dark red contents and/or dark red areas in the stomach. One dam died on gestation day 19 at 50 mg/kg/day but was not considered treatment related. Clinical signs occurring in the dams which died and two survivors at 750 mg/kg/day included head bob, coarse tremors, circling motion and decreased defecation. Body weight loss occurred days 6 - 9 at 750 mg/kg and total body weights were significantly lower days 9 and 12. Fetal weight was lower at the high dose. Possible adverse effect: The mean number of early resorptions was 0.6, 0.8, 1.4* and 1.0 for control through the high dose groups. There were no treatment-related fetal findings for malformations or variations. Maternal NOEL = 375 mg/kg/day (clinical signs, mortality, body weight decrements). Developmental NOEL = 50 mg/kg/day (fetal body weight, resorptions). ACCEPTABLE. (Kishiyama and Gee, 4/4/02).

TERATOLOGY, RABBIT

**** 50275 - 007 128270** Nemec, M. D. "A Developmental Toxicity Study of Tris Nitro[®] in Rabbits" (WIL Research Laboratories, Project No. WIL-129008, 4/22/91) TRIS NITRO[®], 100% ai, was administered via gavage once daily at doses of 0 (deionized water), 10, 30, and 75 mg/kg/day during gestation days 7 through 19 to twenty artificially inseminated New Zealand White rabbits/group. Body weight change and food consumption was reduced for the high dose group during the dosing period. There were clinical signs or effects on pregnancy parameters. Maternal NOEL = 30 mg/kg/day. There were no treatment-related fetal effects. Developmental NOEL = 75 mg/kg/day (no fetal effects). No adverse effects. ACCEPTABLE. (Kishiyama and Gee, 4/3/02).

GENE MUTATION

**** 50275 - 005 073179** Desai, L. S. "TRIS NITRO[®] Brand of 2-hydroxymethyl-2-nitro-1,3-propanediol: Bacterial/Microsomal Plate Incorporation Assay." (Toxikon Corporation, Project No. 88G-0017, March 29, 1988.) TRIS NITRO[®] 100% ai, at concentrations of 0 (acidified water), 0.1, 0.2, 0.3, 0.5, and 1.0 mg/plate with and without rat liver metabolic activation (S9 Mix) was evaluated for mutagenic potential using *Salmonella typhimurium* strains TA 1535, TA1537, TA1538, TA98, and TA100. Concentrations were selected based on a preliminary trial with TA100 at 0.5 to 5 mg/plate. Revertants were reduced at 2 mg/plate and higher. There were triplicate plates in a single trial. Tris-Nitro did not significantly increase the number of revertants. Positive controls were functional. ACCEPTABLE with no adverse effect as conducted. (Kishiyama and Gee, 4/4/02).

**** 50275 - 013 205489** "Evaluation of tris(hydroxymethyl)nitromethane (Tris Nitro[™] Solid CHT) in the mouse lymphoma (L5178Y TK^{+/−}) forward mutation assay." (Linscombe, V. A., M. R. Schisler and D. J. Beuthin, Toxicology & Environmental Research and Consulting, Dow Chemical, Midland, MI, Laboratory ID 011111, March 11, 2002) Tris(hydroxymethyl)-nitromethane (lot. 050101, 101.4%) was assayed with mouse lymphoma L5178Y TK^{+/−} cells for

the induction of mutations. There were duplicate cultures per concentration in each of two trials with and without male rat liver activation. Concentrations in trial 1 without activation ranged from 5.0 to 80 ug/ml, with activation, from 5 to 160 ug/ml. In the second trial, concentrations ranged from 5 to 40 without activation and from 10 to 140 ug/ml with activation. Cytotoxicity at the higher concentrations in all trials prevented plating for mutant frequency. Colonies were sized as large or small. There were concentration-related significant increases in mutant frequency in both trials with and without activation at relative growth > 10%. The increases were predominantly with small colonies. Positive controls were functional. The test was considered positive for genotoxicity. ACCEPTABLE.

CHROMOSOME EFFECTS

50275 - 005 073177 Desai, L. S. "TRIS NITRO[®] Brand of 2-hydroxymethyl-2-nitro-1,3-propanediol: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells." (Toxikon Corporation, Project No. 88G-0204, December 15, 1988.) TRIS NITRO[®], 100% ai, was tested at concentrations of 0, 0.25, 0.50, 1.00, 1.50, and 2.50 mg/ml, with and without metabolic activation (S9 Mix), to determine the potential to induce chromosomal aberrations in Chinese Hamster Ovary cells. Cells were exposed for 2 hours followed by further incubation for 20 hours. Two of the four replicate cultures had BrdU added. Metaphase cells were collected by mitotic shake-off and fixed. Fifty to 100 cells were scored per each of two cultures. The numbers of chromosomal aberrations at various levels of Tris Nitro were not significantly different than the negative control. Positive controls were functional. UNACCEPTABLE (toxicity was not evaluated in the full study for adequacy of testing, types of chromosomal aberrations were not identified and whether gaps were included/excluded in the calculations, fate of all 4 replicates was unclear). Possibly upgradeable with submission of missing data. No adverse effect as conducted. (Kishiyama and Gee, 4/3/02).

** 50275 - 011 205487 " Evaluation of tris(hydroxymethyl)nitromethane in the mouse bone marrow micronucleus test." (P. J. Spencer, Dow Chemical, Toxicology & Environmental Research and Consulting, Lab ID 011210, March 24, 2003) Male and female CD-1(ICR)BR mice were given tris(hydroxymethyl)nitromethane (lot 050101, 101.4%) by gavage with 6/sex/group except for the high doses with 12 each. Males were given doses of 0 (0.5% Methocel), 500, 1000 or 2000 mg/kg/day, on two consecutive days at 24-hour interval with sacrifice at 24 hours after the second dose. Females were given doses of 0, 500, 1000 and 1500 mg/kg/day on the same schedule. Two thousand polychromatic erythrocytes were scored per animal for micronuclei. The PCE/NCE ratios were also recorded. Cyclophosphamide was the positive control. Mortality occurred at the high doses in males (2/12) and females (6/12). Clinical signs and weight loss were also seen at the high doses. There was no statistical significance for mean % MN-PCE in treated females compared with controls. In males, all three treated groups had statistically significantly increased incidence of MN-PCEs compared with concurrent controls but with no dose response, the % being 1.8, 1.7 and 1.5 with increasing dose compared with 0.4 for controls. All values were, however, within the range of recent historical controls for males (range of 0 to 4.0, mean of 1.4 from 7 mean values from recent studies (90 animals)) and there was no dose response. Positive control functioned. The conclusion was that the treatment did not result in an adverse effect. ACCEPTABLE. (Gee, 8/11/03)

DNA DAMAGE

50275 - 005 073178 Desai, L. S. "TRIS NITRO[®] Brand of 2-hydroxymethyl-2-nitro-1,3-propanediol: Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures." (Toxikon Corporation, Project No. 88G-0203, December 24, 1988.) TRIS NITRO[®], 100% ai, was tested at concentrations of 0, 0.313, 0.625, 1.25, and 2.50 mg/ml to measure induction of repair of DNA damage using rat hepatocytes. Concentrations to be used were selected from a cytotoxicity study, ranging from 0.313 to 10 mg/ml. Cytotoxicity was determined by trypan blue dye exclusion. Hepatocytes were exposed for 1 hour to the test article, then incubated for 2 or 24 hours and viability determined. For the UDS assay, hepatocyte cultures were held overnight (following the preliminary cytotoxicity assay), exposed for 1 hour to test article, incubated for 3 hours with ³H-TdR and prepared for autoradiography. There were either three or six replicates (unclear) scored for UDS. Cytotoxicity was reportedly also determined but no concurrent data were presented. The positive control was functional. No significant increase in net nuclear grain counts was reported with Tris-Nitro treatment. The assay was negative under the conditions used. UNACCEPTABLE. Missing information: Justification for 1 hour exposure followed by 3 hours of incubation before fixing, justification for maintaining hepatocyte cultures overnight before the UDS assay, information on whether cytotoxicity was concurrent with the UDS assay, nuclear grain and cytoplasmic grain counts per coverslip with standard deviations, number of coverslips and number of cells scored per concentration. Upgrade questionable. (Kishiyama and Gee, 4/3/02).

** 50275 - 012 205488 " *In vivo/in vitro* unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints with a dose rangefinding assay with TrisNitro[™] Solid CHT." (M. A. Cifone, Covance Laboratories, Vienna, VA, Lab. ID: 23287-0-494 OECD, Dow ID: 011199, April 25, 2002) Male Fischer 344 rats, 4 in vehicle control and 5 at 800 and 1200 mg/kg, were sacrificed at 2-4 hours or 14-16 hours after dosing. Primary hepatocytes were placed into culture, labeled with ³H-thymidine for 4 hours followed by 16 - 20 hours further incubation with 0.25 mM thymidine without tritium. Triplicate cultures were prepared per animal for UDS with a fourth for viability by trypan blue dye exclusion. Net nuclear grain counts were scored for 50 cells per coverslip, three per animal for a total of 150 cells per animal. Dose selection was based on a rangefinding study with 3/sex given 0, 400, 900, 1200, 1600 or 2000 mg/kg with observations for 2 days total. Clinical signs were seen at 1600 and 2000 mg/kg with some mortality beginning at 1200 mg/kg (1/3 males). The three or four animals per group with the best perfusion and/or attachment viability were scored. There was no evidence for the induction of UDS under experimental conditions. ACCEPTABLE. (Gee, 8/11/03).

NEUROTOXICITY

Not required at this time.